# MUTAGENICITY INVESTIGATION OF STEROIDS ON THE AUTOSOME OF DROSOPHILA MELANOGASTER

by

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## Introduction

From the mutational investigations it is known that most of the recessive lethal mutations are small deletions. We know from numerous compounds that they cause by greater incidence delations of different proportions than the X ray. From this is that conclusion logic that the chemicals causing deletions represent a great genetical risk. From the relation between the deletions and the recessive lethal mutations we may suppose that the compound not inducing a lethal recessive mutation also does not cause at the same time a deletion.

The exploration of the compounds causing recessive lethal mutations is also therefore important while these mutations reach the equilibrial state only very slowly and the consequences of the increasing lethal gene frequency appear only protractedly, eventually through several tens or hundreds of generations. When selecting the chemicals to be investigated those compounds should have the priority which influence the essential proportion of the population, in first line those being in the reproductive age. These points of view justified the investigation of the contraceptives especially pointed to the induction of the recessive lethal mutation.

The genetical (so the mutational) effect of the contraceptives is not cleared yet. The investigations carried out in large numbers were limited primarily to the effect onto the cell-division (Jagiello–Lin 1974) respectively to the effect inducing chromosome aberrations but the data are conflicting (Bishun et al. 1976). Since there exists no close correlation between the chromosomal and the gene level damages consequently the results referring to the negative aberration do not mean the harmless nature of the given chemical. Mutational investigations with steroids were carried out only in limited number.

Badr–Badr (1974) observed the increase in DL mutations at high doses. Wallace et al. (1979) did not get an effect referring to mutagenecity as an effect of the Lyndiol treatment. It was unsuccessful to demonstrate any mutagenous peculiarity of the non-steroid synthetic

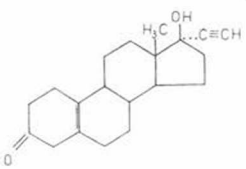
oestrogen; namely the diethilstilboestrol known as being carcinogenous (G l a t t et al. 1979). Two steroidal diamins were found as mutagenous on phages (M a h l e r – B a y l o t 1967) respectively on a system in vitro C h a n g et al. (1975) observed a mutational frequency increased by the 17beta-oestradiol effect.

Our preliminery experiments have shown that after the treatment with contraceptive drugs (being in circulation in Hungary) recessive lethal mutations were isolated in the second chromosome of Drosophila) treated with Infecundin which contains norethynodrel (progesterone) and mestranol (oestrogen).

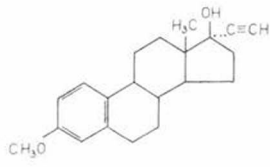
We wanted to characterize these compounds from mutation point of view by the genetical analysis of the mutants isolated after the contraceptive treatment of the flies.

## Materials and methods

We carried out investigations on the Oregon-R strain. We bred the animals on a *Drosophila* medium consisting of yeast, cornflour, raisin, agar-agar at a temperature of 25 °C.




norethynodrel




mestranol

We dissolved the compounds in ethanol of small quantity (2 ml) and mixed them into the substratum in a $3{,}3 \times 10^{-4}$M (noretinodrel) and $1{,}3 \times$ $\times 10^{-5}$M (mestranol) concentrations. The animals grew up on this medium later on we used the hatched males to the detection of the mutation.

We carried out the demonstration of the mutation in the 2nd autosome on the basis as described by A b r a h a m s o n – L e w i s (1971) with the slight modification as follows (Fig. 1):
(The wild phenotype does not occur in the $F_3$ generation if a lethal mutation happened in the 2nd chromosome.)

We grouped the recessive lethal mutants on the basis of a complementational investigation into complementational groups and then we determined the position of the representants of the groups on the chromosome by the method of W a t a n a b e and O s h i n a (1966) (Fig. 2.):

In course of the investigation we crossed the females carrying the marker genes (Sp – Sternopleural – 22.0; Bl – Bristle – 54.8; and L – Lobe – 72.0) with the treated males.

The location of the given lethal was determined on the basis of the decrease in the wild (+) winged (non – Cy) categories.

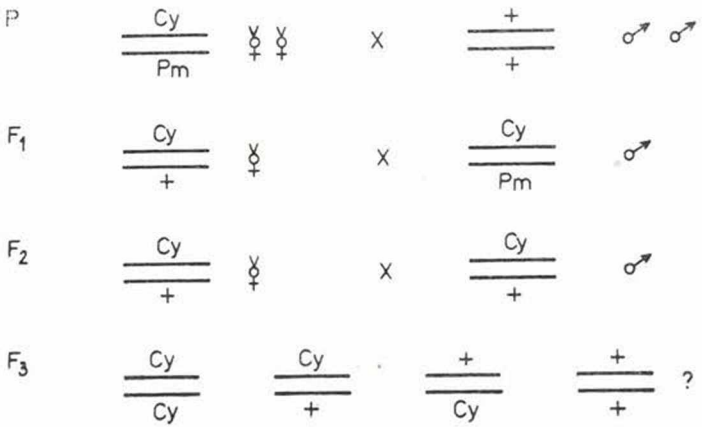

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Fig. 1.

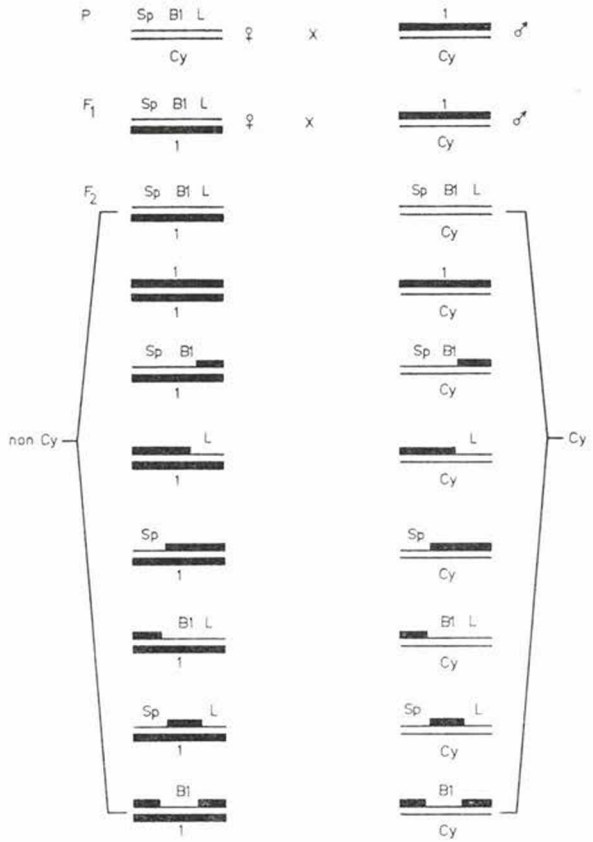
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Fig. 2.

## Results

The frequency of the mutations produced by the synthetical steroids is shown in the Table I.

*Table I*

**Mutational frequency after at the norethyinodrel + mestranol treatment resp, at the controll**

| Series | Noretinodrel + Mestranol (Infecundin) $3{,}3\times10^{-4}$M $1{,}3\times10^{-5}$M | | | Control | | |
|---|---|---|---|---|---|---|
| | Treated chromosomes | Lethal mutations | % | Chromosomes | Lethal mutations | % |
| A – E | 1063 | 48 | 4.32 | 753 | 6 | 0.79 |
| F | 217 | 18 | 8.29 | 221 | 5 | 2.21 |
| G | 181 | 25 | 12.10 | 209 | 24 | 10.30 |

From the data it is visible that the mutational inducational experiments (series A – G) resulted mutation frequency values steadily increasing at the same time the control value is also differing from the 0,5% considered as normal, it is by a magnitudinal order greater than this, thus in the last series it was practically impossible to differentiate the control and the treated groups that is why we excluded the results of this from the further investigations.

We crossed the obtained mutants with each other and arranged them into complementational groups. The crossings representing the complementational investigations are shown in Table II.

*Table II*

**Complementational values of lethal mutations isolated after the norethyinodrel + mestranol (I) resp. norethyinodrel (N) treatment**

+ = complementation exists – = no complementation

| | I1 | I2 | I3 | I51 | N5 | N8 | N10 | N54 | N55 | N60 |
|---|---|---|---|---|---|---|---|---|---|---|
| I1 | – | + | – | + | + | + | + | + | + | + |
| I2 | | – | + | – | – | + | – | + | – | – |
| I3 | | | – | + | + | + | + | + | + | + |
| I51 | | | | – | – | + | – | + | – | – |
| N5 | | | | | – | + | – | + | – | – |
| N8 | | | | | | – | + | + | + | + |
| N10 | | | | | | | – | + | – | – |
| N54 | | | | | | | | – | + | + |
| N55 | | | | | | | | | – | – |
| N60 | | | | | | | | | | – |

We stated by our complementational investigations that the 52 autosomal lethal mutations, isolated after steroidal treatment can be arranged into 6 complementational groups, among which in 5 groups several mutations occur, while the 6th complementational unit is represented by one mutation similarly to the mutation got in the control.

On the basis of the results the question of the chromosomal region specific to the steroids arised what we wanted to clear by the determination of the position of the mutations they occupy on the autosome. The results obtained are represented in table III.

*Table III*

**Distribution of the autosomal recessive lethal mutations into complementational groups and the position of the mutations in the 2nd chromosome**

Lethal mutation isolated by
I = noretinodrel + mestranol treatment
N = lethal mutation isolated by noretinodrel treatment
K = lethal mutation found in the control

| | | | | | | |
|---|---|---|---|---|---|---|
| N – 8 | I – 97 | I – 1 | K | I – 81 | I – 80 | I – 2 |
| N – 53 | | I – 3 | | I – 82 | I – 92 | I – 5 |
| N – 54 | | I – 4 | | I – 83 | I – 93 | I – 8 |
| N – 59 | | I – 7 | | I – 84 | I – 94 | I – 11 |
| | | I – 9 | | I – 85 | I – 95 | I – 51 |
| | | I – 10 | | I – 86 | I – 96 | I – 52 |
| | | I – 12 | | I – 87 | | I – 53 |
| | | | | I – 88 | | N – 1 |
| | | | | I – 89 | | N – 2 |
| | | | | I – 90 | | N – 3 |
| | | | | I – 91 | | N – 4 |
| | | | | | | N – 5 |
| | | | | | | N – 6 |
| | | | | | | N – 7 |
| | | | | | | N – 9 |
| | | | | | | N – 10 |
| | | | | | | N – 51 |
| | | | | | | N – 52 |
| | | | | | | N – 55 |
| | | | | | | N – 56 |
| | | | | | | N – 57 |
| | | | | | | N – 58 |
| | | | | | | N – 60 |
| map position 56.8 | | 63.2 | 63.4 | 65.3 | 66.2 | 67.6 | 69.3 |

As it can be seen from the table, the lethal genes investigated by us are located between the B1 and L markers, they are in the vicinity of the centromere not far from each other (between the 63 – 69 map-units). Only the group represented by the N 8 mutant differs from this which can be found most proximate to the centromere at the 56,85 map-unit. The representant of the lethal genes occurring in the control (K) is to be stressed separatedly which can be localized also to the region mentioned previously (at the 65.3 map-unit).

## Discussion

We observed the induction of recessive lethal mutations in the 2nd autosome of the Drosophila after the administering the synthetical steroids noretinodrel and mestranol. These compounds did not induce at the same time mutations on the sex chromosome (P a r á d i 1981.)

This surprising phenomenon can be interpreted in several ways. It is imaginable that the steroids are bound to specific chromosomal regions and the selective effect is the consequence, of this. In mammals this is obvious since these are matterials of gene-activating.

Since we carried out our experiments on insects as model animals, the question arises how do the steroids get into nucleus of the cell ?

Referring to that what kind of physiological as well as genetical effect the mammalian steroid hormones induce on insects, only few investigations are so far known. Cortisol for instance inhibits in Tenebrio the growth but the moulting cycles remain unaltered, which indicates that the endogenous hormone production did not change (M o r d u e 1967).

From the mutational point of view the entering of the hormone into the nucleus of the cell is the critical step since according to suppositions (S h i e l d s 1976) there occur on the DNS non-specific binding places referring to the hormone-receptor complex, thus the steroid penetration in is capable to react with the DNS. The problem of the penetration in to the nucleus could be clarified by labelled steroids.

The mutational investigations of steroids is also grounded by the close correlation between carcinogenicity and mutagenicity. The relatively high mutational frequency stated by us after the steroidal treatment and the arrangeability of the mutants only into complementation groups of relatively small number arised the question of the existence of regions specific to steroids on the 2nd autosome. The determination of the allocation of the mutations has shown that close to the centromere and not to far from each other they take position.

From other investigations in contrary it is known that the lethals occurring in the nature are not positioned on the autosome random, the centromere region carries more mutations than excepted (G o l u b o v s k i 1971, S p i e s s et al. 1963), within this the maximal occurrence frequency is found between the map-units 50 – 65 (W a t a n a b e – O s h i n a 1966). The fact furthermore that the centromere region can be qualified as a so called low crossing over region means that between the crossing over frequency and the map-distance is the incorrespondance great, by this a map-deformation hence an inexactness in this region can be observed, which supports that supposition that the lethals investigated by us are allocated in a narrow region of the chromosome. This region carries also lethals generated spontaneously, in other words if it could not be considered as a hot spot but anyhow as an instabile region, by this it is probable that it is *not a steroid specific region.*

This means also that the cause of the steadily increasing mutational frequency experienced in course of the investigation should not be searched

in the steroid effect. This is also supported by our already mentioned observation connected to the X chromosome (P a r á d i 1981), in course of which we found the investigated steroids from the point of view of mutation as inactive on the *Drosophila melanogaster*.

## Summary

Among the synthetic steroids occurring in the contraceptive drugs we paid attention to the mutational effect of the norethyinodrel (progesteron and the mestranol estrogen) on the 2nd autosome of *Drosophila melanogaster*.

We found that:

- the recessive lethal mutations isolated after the steroid treatment can be arranged to 6 complementational groups.
- the mutations can be localized to the environ of the centromere in which region spontaneous mutation occur too. The region can be considered as instabile thus the specifity of the steroid is not probable therefore the mutations observed were most probably not generated as the effect of the steroids. This is supported by our previous observation according to which the investigated steroids do not induce recessive lethal mutation on the X chromosome.

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